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Future directions for therapeutic strategies in post-ischaemic vascularization: a position paper from European Society of Cardiology Working Group on Atherosclerosis and Vascular Biology

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Abstract

Modulation of vessel growth holds great promise for treatment of cardiovascular disease. Strategies to promote vascularization can potentially restore function in ischaemic tissues. On the other hand, plaque neovascularization has been shown to associate with vulnerable plaque phenotypes and adverse events. The current lack of clinical success in regulating vascularization illustrates the complexity of the vascularization process, which involves a delicate balance between pro- and anti-angiogenic regulators and effectors. This is compounded by limitations in the models used to study vascularization that do not reflect the eventual clinical target population. Nevertheless, there is a large body of evidence that validate the importance of angiogenesis as a therapeutic concept. The overall aim of this Position Paper of the ESC Working Group of Atherosclerosis and Vascular biology is to provide guidance for the next steps to be taken from pre-clinical studies on vascularization towards clinical application. To this end, the current state of knowledge in terms of therapeutic strategies for targeting vascularization in post-ischaemic disease is reviewed and discussed. A consensus statement is provided on how to optimize vascularization studies for the identification of suitable targets, the use of animal models of disease, and the analysis of novel delivery methods.

Keywords

Post-ischaemic angiogenesis • Pre-clinical studies • Gene and cell delivery • Clinical trials

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1. Basic principles: vascularization, angiogenesis, and arteriogenesis

Vasculogenesis describes the coalescence of mesoderm-derived angioblasts into the first primitive blood vessels.¹ The process was first observed in quail embryos^{2,3} and subsequently, shown to be conserved in other vertebrates including mouse^{4,5} and zebrafish.^{6,7} These studies revealed many similarities not only between the morphogenetic processes of early blood vessel formation, but also between the molecules co-ordinating these processes.⁸ Several signalling pathways such as Notch^{9,10} and Sonic Hedgehog,¹¹ were shown to influence the early differentiation of arterial and venous endothelial cells (ECs) from angioblasts. Vasculogenesis was initially thought to be limited to the embryo, but current understanding is more nuanced. Early embryonic angioblasts and haemoblasts share a very similar gene signature and haematopoietic stem cells (HSC) and ECs display considerable plasticity.^{12,13} Notably, HSC can be differentiated into ECs,¹³ and these progenitors have shown therapeutic potential in several clinical and pre-clinical settings.¹⁴

Angiogenesis is the creation of new vessels from pre-existing ones.¹⁵ Hypoxia is one of the key drivers of the process. It activates ECs to become more motile and protrude filopodia. Further angiogenic factors such as vascular endothelial growth factor (VEGF) strongly dilates small arteries and capillaries, which is the primary mode of VEGF action at low concentrations (intussusception angiogenesis). At high concentrations of VEGF, sprouting angiogenesis is the preferred mode of action.¹⁶ To prevent ECs moving *en masse*, a particular type of ECs, known as tip cells, are selected to lead the advance.¹⁷ Neighbouring cells assume an ancillary role as stalk cells, which divide to elongate the new vessel and establish a lumen. This specification of tip and stalk cells is governed by the Notch signalling pathway.^{18,19} The establishment of flow in newly formed vessels leads to mechanical signals (shear stress) that feedback to reduce angiogenic sprouting, thereby preventing excessive vascular growth.^{20,21}

Once stenosis in a large main artery becomes haemodynamically significant, the elevation of shear stress against the wall of these arterioles induces their enlargement. This is described as *arteriogenesis*. The collateral circulation may subsequently develop into a functional vascular structure to ensure regional perfusion after the ischaemic event, thus protecting the tissues against necrosis. Simultaneously, arterioles, venules, and arteriovenous anastomoses are formed, following the production of smooth muscle cells and of the extracellular matrix (ECM), which consolidates the walls of these vascular structures.²²

2. Neo-vascularization: physiology and pathophysiology

2.1 Post-ischaemic vascularization

After the onset of ischaemia, cardiac or skeletal muscle undergoes a continuum of molecular, cellular, and extracellular responses that determine the function and the remodelling of the ischaemic tissue. Hypoxia-related pathways, the alterations in immunoinflammatory balance, as well as changes in haemodynamic forces within the vascular wall trigger vasculogenesis, angiogenesis, and arteriogenesis, which act in concert to establish a functional vascular network in ischaemic zones.²³

The principal signalling pathway induced by hypoxia involves activation of hypoxia-induced factor (HIF1 α), which induces the expression of a set of genes appropriate to respond to this situation. Indeed, HIF1 α controls the expression of numerous major players involved in angiogenesis and

vascular remodelling including VEGF. Moreover, the target genes of HIF1 α are involved in metabolism, erythropoiesis, pH homeostasis, and autophagy.²⁴

During ischaemia, inflammatory cells release angiogenic factors (e.g. VEGF) and cytokines (e.g. TNF α), which decrease EC junctions and enhance vascular permeability to promote the recruitment of inflammatory cells.^{25,26} Consistent with this relationship between angiogenesis and inflammation, several molecules that regulate inflammation have been implicated in new vessel formation.²³ Changes in haemodynamic forces (mechanical forces linked to pressure and flow rate) occurring in collateral vessels in response to arterial occlusion also contribute to post-ischaemic vascularization.²⁷ Recent studies suggest that flow dynamics control the activation of HIF1 α ²⁸ and the localization of sprouting in vessels.²⁹ The location is not determined by on highest VEGF concentration, but by a combination of VEGF and biomechanical signals.³⁰ Thus, shear-induced mechanism appears to override pro-angiogenic signals such as VEGF.³¹ These pathways can also participate in vascular pathology; for example, the mechanosensitive transcription factor TWIST1 promotes angiogenesis in the embryo and is also required for plaque formation in atherosclerosis models.²¹

In patients with ischaemic diseases in the presence of comorbidities such as diabetes, hypertension, and obesity, most of the cellular and molecular mechanisms involved in the activation of vessel growth and vascular remodelling are markedly impaired.²³ Thus, in the last decades, stimulation of vessel growth has emerged as a novel therapeutic option in patients with ischaemic diseases.³²

2.2 Vascularization of atherosclerotic plaques

Under physiological circumstances, microvessels originate from the adventitia and provide the media of large arteries with oxygen and nutrients.³³ However, microvessels in atherosclerotic plaques have been implicated in progression of the disease and adverse outcomes.

It is postulated that plaque angiogenesis is driven by plaque hypoxia and inflammation.^{34,35} In experimental models, plaque angiogenesis has been induced by stress,^{36,37} treatment with pro-inflammatory mediators,³⁸ pro-angiogenic growth factors,³⁹ and viral gene delivery of pro-angiogenic factors^{40–44} and was shown to increase plaque burden. Besides an increase in the number of microvessels, the physiological properties (quality) of the microvessel are also associated with risk for human plaque rupture. Microvessels of ruptured plaques in coronary arteries displayed detachments of the endothelial junctions, endothelial membrane blebs and a thin or absent endothelial basement membrane, and surrounding pericytes were found to be absent in a majority of microvessels in ruptured plaques.⁴⁵ These ultrastructural characteristics suggest vascular leakage,⁴⁶ which might be responsible for increased extravasation of immune cells and deposition of lipids and red blood cells in the plaques.^{47–49} Therefore, these microvessels are thought to represent one of the main sources of intra-plaque haemorrhage, in addition to healed thrombi.⁵⁰

3. Therapeutic vascularization

3.1 Growth factors, cells, and non-coding RNA therapies

Multiple different approaches have been used to promote vascularization of ischaemic tissues.

Table 1 Gene therapy in post-ischaemic vascularization

Growth factors	Models	Outcomes	References
VEGF	Pig MI	Increase neoangiogenesis, improved regional myocardial function, and myocardial perfusion	51
bFGF	Pig MI	Enhanced arteriogenesis within the ischaemic zone	52
HGF	Rabbit HLI	Increase of blood flow and arteriogenesis	53
Ang-1	Mouse MI	Increase in capillary density, reduction in infarct sizes, and increase heart performance	54
IGF-1	Mouse MI	Increase in capillary density and increase heart performance	55
Disease/patient number	Growth factor/vector/delivery	Primary outcomes	Trial
PAD/60	FGF-2/SeV/ i.m.	Walking performance	NCT02276937
PAD/500	HGF/Pl/i.m.	Time to major amputation	NCT02144610
MI/41	VEGF-A116A/Ad/i.my	Time to 1 mm ST depression during exercise stress testing	NCT01757223

Ad, adenovirus; HLI, hind-limb ischaemia; i.m., intramuscular; i.my., intramyocardial; MI, myocardial infarction; PAD, peripheral artery disease; Pl, plasmid; SeV, sendavirus.

3.1.1 Growth factors

Growth factors have been applied for therapeutic angiogenesis including VEGF,⁵¹ basic fibroblast growth factor (bFGF),⁵² hepatocyte growth factor (HGF),⁵³ Angiopoietin 1 (ANG-1),⁵⁴ and insulin-like growth factor (IGF-1)⁵⁵ (Table 1). Pre-clinical studies in animal models using individual angiogenic factors have showed significant improvements in clinically relevant endpoints such as increased regional perfusion, improved exercise tolerance and tissue energy metabolism, improved myocardial function, and protection against ischaemic damage.⁵⁶ Among these, VEGF, bFGF, and HGF are the best studied and have reached human clinical trials (Table 1). However, apart from demonstration of increased vascularity, very few results with clinical significance have been obtained.

VEGF is a critically important regulator of physiological angiogenesis during growth, healing and in response to hypoxia. VEGF is up-regulated by HIF1 α more than any other inducible angiogenic factor during ischaemia. However, when administered alone, VEGF can increase endothelial permeability, which leads to the formation of leaky capillaries and tissue oedema.⁵⁷ Platelet Derived Growth Factors (PDGF) can help stabilize nascent blood vessels by recruiting mesenchymal progenitors, and co-delivery of VEGF and PDGF has been shown to lead to early formation of mature vessels in animal models.⁵⁸ Basic fibroblast growth factor is among the first discovered angiogenic factors to have both angiogenic and arteriogenic properties, which may facilitate formation of a mature blood vessel network.⁵⁹ The HGF family induces potent angiogenic responses by binding to the c-MET receptor, which is expressed on ECs, vascular smooth muscle cells, and HSC. HGF is known to have mitogenic, angiogenic, anti-apoptotic, and anti-fibrotic activities in various cells.⁶⁰ Clinical trials of SDF-1 in critical limb ischaemia (CLI) patients are underway and a better understanding of the mechanisms of chemokines, especially SDF-1, is crucial in filling the missing link in growth factor studies in therapeutic angiogenesis.⁶¹

3.1.2 Cell therapy

Cell-based therapy has been demonstrated to have the capability of tissue repair in many animal studies and in ongoing clinical trials (Table 2). Cell transplantation in ischaemic tissue may attenuate severity of tissue damage and accelerate the regeneration process. Genetic modification, pre-conditioning, and tissue engineering have been applied to improve the efficacy of stem cell therapy.⁶⁵ Since the first pilot clinical study to evaluate treatment of peripheral vascular disease with stem cell therapy

in 2002, over 50 clinical studies have been reported with stem, progenitor, and stromal cells⁶⁶ (Table 2).

Therapeutic details such as patient selection, effective cell selection and processing, optimal dosage, and delivery route are constantly improved. Studies have included patients of varying periphery artery disease (PAD) severity. However, most of clinical trials have primarily focused on CLI patients in small Phase I or II studies.⁶⁶ A variety of cell types have been studied as potential PAD treatments including unselected bone marrow mononuclear cells (BM-MNC) or peripheral blood MNC (PB-MNC), marker-specific cells selected from the marrow or blood, mesenchymal stem cells (MSCs), and adipose tissue-derived regenerative cells.⁶⁷ In clinical studies of neovascularization considerable progress in the use of adult stem cells for cell transplantation has been made using HSC, bone marrow-derived dendritic cells, MSC, and endothelial progenitor cells.¹⁴ Neovascularization in infarcted heart can be mediated by the incorporation of vascular progenitor cells into the capillary or by the paracrine factors released from stem cells and progenitor cells. In relation to the effectiveness of the use of adult stem cells for cell transplantation, the variability in the reported findings may be partly explained by differences in the delivery methods, treatment logistics, and target diseases.¹⁴

3.1.3 Non-coding RNA therapy

Short (microRNAs; miRNAs) or longer [long non-coding RNA (lncRNAs)] non-coding RNAs play important roles in several physiological and pathological conditions such as cancer and cardiovascular diseases including atherosclerosis.⁶⁸ Emerging data show that several miRNAs are linked to both adaptive and maladaptive vascular remodeling processes. Mir-126, one of the most abundantly expressed microRNAs in ECs, has a pro-angiogenic as well as anti-atherosclerotic role⁶⁹ and the systemic delivery of miR-126 mimics rescued EC proliferation at vulnerable sites and inhibited atherosclerotic lesion progression.⁷⁰ On the other hand, the 17-92 miRNA cluster is anti-angiogenic but pro-atherosclerotic. Recent studies described that the endothelial-specific deletion of miR-17-92 in mice enhanced arterial density and improved post-ischaemia blood flow recovery.⁷¹ Notably, miR-503 expression is increased in ischaemic limb muscles and ECs of diabetic mice. Inhibition of miR-503 by adenoviral delivery to the ischaemic adductor muscles of diabetic mice corrected diabetes-induced impairment of post-ischaemic angiogenesis and blood flow recovery.⁷² Even though the

Table 2 Cell therapy in post-ischaemic vascularization

Cell lines	Models	Outcomes	References
BM-derived haematopoietic stem cells (CD34 ⁺)	Pig MI	Greater vessel densities and higher expressions of bFGF and SDF-1	⁶²
BM-derived mesenchymal stem cells	Pig MI	Reduction in infarct size and increases in ejection fraction	⁶³
Cardiac stem cells	Pig MI	Reduction in infarct size and increase in contractility	⁶⁴
Disease/patient number	Cell line/delivery	Primary outcomes	Trial
MI/142 MI/55	Cardiac stem cells/i.c.	Infarct size by MRI Safety as measured by death and MACE in 12 months	ALLSTAR trial (NCT01458405). CAREMI trial (NCT02439398)
MI/3000	Autologous BM-derived mono-nuclear cells/i.c.	Time from randomization to all-cause death	BAMI trial (NCT01569178)
Ischaemic heart failure/315	BM-derived mesenchymal stem cells/i.c.	Efficacy between groups post-index procedures	CHART-1 (NCT01768702)CHART-2 (NCT02317458)

i.c., intracoronary; MI, myocardial infarction.

functions of individual microRNAs in angiogenesis are not yet completely elucidated, because a single microRNA could regulate several growth factors at the same time, miRNA-derived therapy could replace single-factor angiogenic gene therapy.⁷³

3.2 Gene and cell delivery

Delivery of therapies into the myocardium has been a major challenge over the past decade. Efficient therapeutic approaches developed in animal models have not been successful in human clinical trials because gene and cell transfer efficiency in cardiac muscle has been too low.^{56,74} Several factors contribute to this problem: the human heart is a very large muscle when compared with mice and rats and vectors or cell solutions cannot easily penetrate deep into the myocardium. The adeno-associated virus for instance, bind tightly to heparansulphate proteoglycans and they do not easily escape from the intraluminal space into the myocardium.⁷⁵ In previous trials, intracoronary injections, intramyocardial injections from the left ventricle, and intramyocardial injections during thoracotomy or bypass surgery have been tested. However, because occluded coronary arteries do not get adequate perfusion, fail to deliver substances into the ischaemic areas. Thus, it is not surprising that intracoronary injections have had poor success for gene and cell delivery.

3.2.1 Mechanical delivery

Intramyocardial injections lead to better transduction efficiencies but diffusion of viral vectors in the myocardium is still limited and the binding to ECM components further limits vector spreading in the myocardium. Protein, such as VEGF-A₁₆₅, delivered by transgenes, bind strongly to heparansulphate proteoglycans, which reduces their diffusion in ischaemic and fibrotic myocardium. Similar obstacles exist for successful cell delivery into the myocardium. Intracoronary injections seldom lead to viable, engrafted cells in the heart. Intramyocardial injections cause significant mechanical stress on the cells during injections. Most cells seem to die within hours or during the first days and paracrine factors seem to contribute to the potential therapeutic effects.^{76,77} For applications such as myocardial ischaemia, local targeted injections based on electromechanical mapping,⁷⁸ or blood flow measurements using positron

emission tomography⁷⁸ have recently improved the situation and targeted injections into hibernating myocardium can now be achieved with 10–20% efficiency around the needle track. Multiple injections are still needed to cover larger areas in ischaemic myocardium. To improve myocardial function in heart failure, the effects of gene or cell transfer should be very global to transduce as many cardiomyocytes as possible. At the moment, this can be achieved with some vectors in mice⁷⁹ but in larger animals and humans wide spread gene expression after any delivery method still remains a very challenging task.⁸⁰

3.2.2 Non-viral delivery

Several methods of non-viral gene transfer have been utilized to deliver genes of interest to ischaemic tissues to stimulate therapeutic angiogenesis. Genes encoding pro-angiogenic proteins have been administered by cationic polymers, lipids, liposomes, and three-dimensional scaffolds.⁸¹ Targeting strategies using polymers or lipids modified with specific ligands for the receptors on target tissues could improve the efficacy of current gene delivery systems by facilitating cellular uptake of genes via receptor-mediated endocytosis.⁸² Gene delivery using lipid formulations has been applied in ischaemic tissues for therapeutic angiogenesis. Jeon et al.⁸³ reported that VEGF-A gene delivery using heparin-conjugated Polyethylenimine significantly up-regulated VEGF-A expression, resulting in extensive neovascularization in mouse ischaemic limbs. Nanoparticles composed of biocompatible and biodegradable polymers [e.g. poly (lactic-co-glycolic acid; PLGA)] are considered to serve as gene carriers for the treatment of ischaemic tissues due to the efficient delivery mechanism and low toxicity.⁸⁴ A novel concept of involving a biodegradable gelatin hydrogel carrying a sustained-release system of bFGF was studied in patients with CLI.⁸⁵

3.3 Animal models

Models to investigate post-ischaemic angiogenesis have been established in rodents and larger animals such as rabbits, pigs, or dogs (Table 3). They exhibit considerable variation because each species differs in the extent of naïve vascularization and thus reacts differently to vascular growth stimuli (Figure 1). To make things more complicated, within one

Table 3 Large animal models of post-ischaemic vascularization

Models	Readout	References
Left anterior descending coronary artery ligation	Myocardial infarct size	86
Femoral artery ligation	Hind-limb perfusion	16
Femoral artery excision	Hind-limb perfusion	87
Coronary stenosis	Myocardial infarct size	88,89
Left anterior descending coronary artery ligation	Myocardial infarct size	90
Femoral artery ligation	Hind-limb perfusion	91
Ameroid constrictors and coronary artery ligation	Myocardial infarct size	92
Ameroid constrictors	Myocardial infarct size	93

animal species, different strains show distinct naïve vascularization and even show opposite reactions.⁹⁴

So far, most studies have been performed in mice, because of the availability of a wide range of genetic knockout strains and the ease of introducing new genetic manipulations, including knock-in and temporal or tissue-specific manipulations. Moreover, the breeding is relatively fast and less expensive than experimentation with large animals and data obtained in mouse models are still necessary to justify experiments in large animal.

A commonly used method in mice to induce post-ischaemic angiogenesis is the hind-limb ischaemia model, which is based on ligation of the femoral artery.⁹⁵ Compared with the coronary or carotid artery, the femoral artery is easier to access, and the method is accompanied by lower mortality rates. Moreover, live imaging of blood flow in ischaemic areas can be easily performed by laser Doppler imaging. Nevertheless, many of the mechanisms underlying neovascularization in response to ischaemia in peripheral arteries are not directly transferable to angiogenic processes in the heart. Experimental models of cardiac ischaemia are based on transient or permanent occlusion of the left descending coronary artery, induced by a highly invasive surgical procedure requiring thoracotomy. Moreover, *in vivo* imaging of coronary arteries by for instance intravital microscopy is complicated by the rapid movements due to cardiac and respiratory cycles.⁹⁶

Rat models are also frequently used due to the ease of breeding and their extended lifespan. The methods and readouts normally applied do not differ essentially from those used in mice. Their major advantage compared with mice, therefore, lies in their size, without improving translatability into humans. Moreover, larger animals require a longer time to restore vessel function by neovascularization. Of course, this is an oversimplification, but it partly explains why larger animal models are often regarded to have added value for translation of angiogenic therapies into human medicine.

For a long time, the dog,^{92,93} together with the rabbit,^{16,86} were the animals of choice for investigation of neovascularization. Amongst other reasons such as easy handling, dogs are well known for their extended myocardial vascularization that allows performing coronary artery occlusions with low complication rates. Much of our current knowledge on the role of various angiogenic and arteriogenic growth factors is based on experiments performed in dogs. However, ethical considerations

have led to a significant decrease in the use of dogs for animal experimentation.

The occlusion pathophysiology and tissue recovery that occur after an acute arterial ligation are very different in animal models than in human chronic ischaemic diseases. Experimental acute vessel occlusion results in an immediate vascular response in animals, which reflects the situation in a limited subgroup of patients (such as young patients with traumatic injuries), who require immediate medical interventions and are not typically enrolled in angiogenic therapy clinical trials. Another crucial difference between the experimental models and patients is that the patients, owing to their comorbidities, do not have sufficient growth of collaterals, showing decreased endogenous angiogenic stimuli and reduced angiogenic signalling.³²

The search for an adequate replacement with potentially even higher translational value has resulted in an increasing number of pig models. Hind-limb ischaemia in pigs can be safely performed without leading to limb necrosis.⁹¹ In contrast, the pig was long considered to have insufficient capabilities to compensate for coronary ischaemia by neovascularization.⁹⁰ In the past decade, however, several groups succeeded in establishing also pig coronary neovascularization models by inducing progressive coronary stenosis rather than acute occlusions.^{88,89}

3.4 Clinical trials for therapeutic vascularization: change of perspectives

3.4.1 Endpoints

Ongoing clinical gene and cell therapy trials have been reviewed elsewhere.^{74,97} In most ongoing trials, very stringent endpoints have been selected such as overall mortality, major adverse cardiovascular events (MACE), improvement in exercise test, or various quality of life endpoints. However, since most gene and cell therapy trials are still quite small when compared with large pharmaceutical Phase II/III trials, they do not have sufficient statistical power to capture endpoints such as overall mortality or MACE. For example, small Phase I and Phase II clinical trials for CLI have shown that cell-based therapies are safe and improve wound healing, but the trials were not large enough to detect any improvements in delaying amputation.⁶⁷

Ideally, functional readouts based on imaging such as positron emission tomography or magnetic resonance imaging should be obtained in parallel with hard clinical endpoints to validate the biological effects of the intervention along the way. It would be especially important to measure functional improvements in the myocardial function and extend analysis to various sensitive imaging and metabolic measurements. In cancer trials for example, it is well accepted that drugs can be approved based on imaging-derived complete or partial responses and/or timelines to recurrence even though there are no effects on survival or mortality.⁹⁸ In addition, it is likely that only some patient populations will be responding positively to gene and cell therapies and therefore it would be important to identify biomarkers, which could differentiate responders from non-responder populations.⁹⁹

3.4.2 Patient populations

So far, while non-controlled, non-randomized gene and cell therapy trials in cardiovascular diseases have provided positive outcomes, most randomized, controlled, blinded studies have not achieved any clinically relevant effects in heart and limb muscles.¹⁰⁰ In multi-centre studies, heterogeneity in patients and different cell preparations and products can influence the efficacy of cell therapy.¹⁰¹ In addition, meta-regression showed that refinements in endovascular and surgical techniques leading

Table 4 Therapeutic strategies to reduce plaque angiogenesis

Animal model	Treatment	Duration	Readout	Effect on plaque size	Intra-plaque angiogenesis	Adventitial angiogenesis	References
ApoE ^{-/-} LDLr ^{-/-} mouse	Thalidomide	39 weeks chow	μCT	↓	ND	–	104
Collar placement + LDLr mouse	VEGFR2 vaccination	Not clear	Histo	↓	–	Present	109
ApoE ^{-/-} mouse	TNP-470	20 weeks HCD	Histo	↓	–	–	105
Collar placement + LDLr mouse	Tie2 vaccination	8 weeks HCD	Histo	↓	–	↓	108
Rabbit	Bevacizumab	3 weeks HCD	Histo	↓	–	↓	107
Balloon angioplasty pig	Endostatin (Endostar)	12 weeks HCD	Histo	↓	ND	↓	110

to improved limb salvage reduces the potential impact of cell therapy.¹⁰¹ Therefore, future cardiovascular gene and cell therapy trials should focus more on randomized, blinded and controlled study designs where less severely affected patients are treated when compared with so called no-option patients, which have been frequently targeted in previous non-randomized trials. It is likely that these no-option patients have already lost at least some of their regenerative capacity, and therefore, are not optimal for testing new biological therapeutic approaches.

3.4.3 Growth factor development

To achieve better outcomes, an optimal profile of growth factors should be identified for clinical testing since some of the previously tested factors such as VEGF-A, are problematic because they increase vascular permeability and thrombosis. Instead, growth factors with more appropriate signalling kinetics for improving cardiac condition should be taken into clinical testing. A possible example is VEGF-D, which is both angiogenic and lymphangiogenic, and therefore, can improve fluid drainage from myocardium after inducing angiogenic effects. Signalling kinetics for VEGF-D are also longer lasting than VEGF-A. Therefore, it may be better suited for therapeutic applications than the previously tested growth factors. Recent Phase I/IIa clinical trial results in refractory angina patients have indeed supported this approach. The trial results showed improved myocardial perfusion reserve in the treated ischaemic, hibernating myocardium 1 year after the treatment.¹⁰² Also, the trial suggests that patients with high Lp(a) benefit most from the adenovirus VEGF-D therapy. Therefore, we can expect improved therapeutic applications in the future after learning important lessons from the previous trials.

4. Vascularization of atherosclerotic plaques

The therapeutic benefits of enhanced vascularization of ischaemic tissues in ischaemic tissues contrasts with the effects of vascularization in atherosclerotic lesions, which can enhance plaque burden and also promote plaque rupture^{45,103} potentially leading to myocardial infarction or stroke.

4.1 Therapies

Investigations using animal models have shown that inhibiting vascular growth factors can preserve vascular integrity and reduce plaque angiogenesis. Notably, most of the intervention strategies to manipulate angiogenesis in atherosclerosis have been restricted to mouse models using molecules such as thalidomide,¹⁰⁴ TNP-470,¹⁰⁵ angiostatin,¹⁰⁶ monoclonal antibody anti-VEGF-A,¹⁰⁷ and VEGFR2¹⁰⁸ or Tie2 inhibitors¹⁰⁹ (effects summarized in Table 4). However, since VEGFs are

involved in important physiological processes, it is not surprising that multiple trials with VEGF inhibiting compounds show also cardiovascular harmful effects.¹¹¹

4.2 Animal models

Many studies of atherosclerosis use murine models, however, there are several limitations in their applicability to analyse plaque vascularization (Table 5). Notably, atherosclerotic plaques developing in hypercholesterolaemic murine models contain fewer microvessels than human atherosclerotic plaques. The reason for this remains uncertain, but it may be due to differences in the transport of oxygen between human versus murine atherosclerotic plaques, ECM turnover and different biomechanics between mice and human.¹¹⁵ A role for ECM was implicated by studies of knockout mice lacking collagen XVIII, which had enhanced intra-plaque vascularization in response to hypercholesterolaemia compared with controls.¹⁰⁶ This was more pronounced in ApoE fibrillin double knockout mice,¹¹² suggesting that lack of proper ECM components in the media and plaque might mediate angiogenesis. Besides ECM degradation, different biomechanical properties between mice and human might also explain the lack of plaque angiogenesis.^{116,117} Lower fibrotic material stiffness (cellular and hypocellular) and a fundamental difference in plaque morphology (dome-like) together with a smaller vessel size as well as lower peak cap stress are present in murine compared with human plaques.¹¹⁷ In addition, tissue contraction and deformation have been shown to induce VEGF-A expression.¹¹⁸ Lower biomechanical stresses might account for lower VEGF-A levels in mice versus humans. Indeed, ruptured human plaques express higher levels of VEGF-A compared with stable plaques.¹¹⁹ In murine atherosclerosis, experimental overexpression of VEGF-A increased signs of plaque vulnerability,³⁹ showing that endogenous VEGF-A expression is not sufficient to evoke signs of plaque rupture.

Another limitation relates to the site of microvessel formation. While a minority of studies report intra-plaque angiogenesis in murine atherosclerosis models, most focus on plaque-associated vasa vasorum of the adventitia as a surrogate for intra-plaque microvessels (Table 5). This is an important caveat because although adventitial vasa vasorum growth may precede atherosclerotic plaque development,^{120,121} plaque rupture has been linked with increased intra-plaque angiogenesis rather than an increase in adventitial vasa vasorum in humans.⁴⁵ Thus far, this discrepancy limits the extrapolation of murine adventitial angiogenesis as an outcome parameter to human studies.

Moreover, several methodological limitations hamper the comparability of murine and human studies. Firstly, while murine models usually examine on various regions (e.g. aortic root, ascending aorta, descending aorta, brachiocephalic artery, and carotid artery) they often ignore other

Table 5 Modelling effects of enhanced angiogenesis on mouse atherosclerotic plaque

	Mouse model	Treatment	Duration	Readout	Effect on plaque	Intra-plaque angiogenesis	Adventitial angiogenesis	References
Short time diet	ApoE ^{-/-}	VEGF-A	7, 6, 5 weeks HCD	Histo	↑	↑	ND	39
	ApoE ^{-/-}	VEGF-A	7, 6, 5 weeks HCD	Histo	↑	↑	ND	39
	LDLR ^{-/-} ApoB38 ^{-/-}	Time + VEGF-A, VEGF-B, VEGF-C, VEGF-D gene transfer	12 weeks HCD	Histo	=	–	=	44
	ApoE ^{-/-} Coll XVIII ^{-/-}	Coll XVIII KO	24 weeks HCD	Histo	↑	↑	↑	106
	ApoE ^{-/-} Fbn1 C1039G ^{+/-}	Fbn1 C1039G ^{+/-} KO	20 weeks HCD	Histo	↑	↑	Present	112
Aged mice and/or prolonged diet time	ApoE ^{-/-}	Time	40–50 weeks chow	Two photon microscopy	–	↑	↑	49
	ApoE ^{-/-}	bFGF	(I) 67–94 weeks chow (II) 12 weeks HCD	Histo	↑	ND	↑	113
	ApoE ^{-/-}	Time	40–96 weeks HCD	Intravital microscopy	–	↑	↑	48
	ApoE ^{-/-} SV129 ^{-/-}	Time + stress + SV129 KO	20 weeks HCD	Histo	↑	↑	ND	36
	ApoE ^{-/-}	Collar Placement + MMP9 gene therapy	Not clear	Histo	=	=	ND	40
Surgical Manipulation	ApoE ^{-/-}	Collar placement + VEGF-A gene transfer	Not clear	Histo	↑	=	ND	40
	ApoE ^{-/-}	Tandem Stenosis	17, 13, 10, 8 weeks HCD	Histo	↑	Present	Present	114
	ApoE ^{-/-}	Wire injury + alternative spliced Tissue Factor gene transfer	6 weeks HCD	Histo	↑	↑	ND	43

Table 6 Large animal models of plaque angiogenesis

Animal Species	Anti/Pro Angiogenic	Treatment	Duration	Readout	Effect on plaque	Intra-plaque angiogenesis	Adventitial angiogenesis	References
Rabbits	Pro	VEGF-A	6 weeks HCD	Histo	↑	Increase but only total CD31 measured not density	ND	39
	Pro	Perivascular Collar + VEGF-A, VEGF-CNC, VEGF-D and VEGF-DNC gene transfer	3 weeks HCD	Histo	↑	ND	↑	41
	Pro	Perivascular Collar + VEGF-E, VEGF-E+ soluble VEGFR2 gene transfer	10 days chow	Histo	↑	ND	↑	123
	Pro	Collar placement (rabbit)+ balloon angioplasty (rat) with VEGF and PR39 gene transfer	9 days (rabbit) and 14 days (rat) chow	Histo	↑	ND	↑	42
	Pro	Watanabe + Alloxan injection to induce diabetes		Histo NMR	↑	Total CD31 not density	ND	124
Pigs	–	PCSK9 knock-in	46 weeks HCD	Histo	–	Present	Present	125

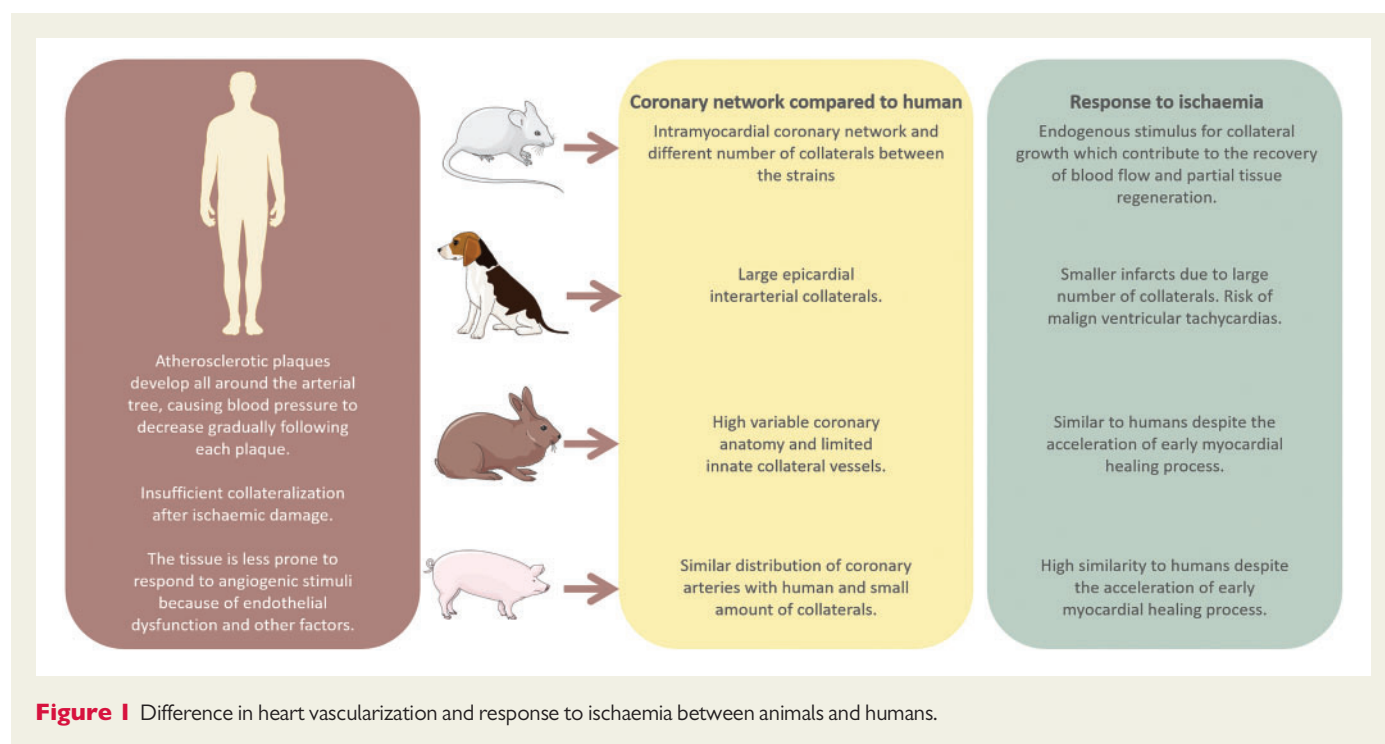


Figure 1 Difference in heart vascularization and response to ischaemia between animals and humans.

clinically-relevant vessels such as the coronary and renal arteries. In addition to this, the parameters measured to assess vascularization vary considerably between studies: for example, microvessel density (number of microvessels per mm^2), microvessel count (per section or per mouse), CD31 positive adventitial area, or vasa vasorum volume have been used (Table 5). Moreover, also the imaging method varied between studies: most of them used histology, but also intra-vital microscopy, two photon microscopy, confocal microscopy, and micro computed tomography have been used to visualize adventitial microvessels (Table 5). Moreover, the experimental design often limits the translatability of the findings. In two studies, induction/manipulation of angiogenesis was started together with atherosclerosis induction,^{104,122} whereas pre-existing plaques represent the treatment target in human atherosclerosis.

In addition to mice and rats, rabbits and pigs have been used to study angiogenesis in atherosclerosis (Table 6). In rabbit models, atherosclerosis was mostly induced by a combination of balloon angioplasty and high cholesterol diet, leading to plaques with a baseline microvascular density between 15 and 80 vessels per mm^2 . In some studies, adventitial angiogenesis was specifically targeted using a hollow perivascular collar together with a relatively short post-operation time of 9–21 days.^{41,42,123,126} Interestingly, induction of diabetes accelerated atherogenesis and intra-plaque angiogenesis in Watanabe heritable hyperlipidaemic rabbits.¹²⁴

In pigs, atherosclerosis was induced by high-cholesterol diet and/or surgical interventions (balloon angioplasty or stenting). However, intra-plaque angiogenesis was not detected in all studies except for one. Here, a genetically engineered Yucatan mini pig was used, which develops hypercholesterolaemia due to pro-protein convertase subtilisin/kexin type 9 (PCSK9) overexpression, when fed a high-cholesterol diet.¹²⁵ The resulting plaques show a human such as morphology including intra-plaque and adventitial angiogenesis. However, data on microvascular density were unfortunately not provided. Practically, larger animal models allow for the use of clinical diagnostic tools such as magnetic

resonance imaging to detect microvessels. Therefore, it will be easier to translate the study results to the human situation.

5. Consensus statement

In this article, the ESC Working Group for Atherosclerosis and Vascular Biology provides guidance for the development of treatments to target the vasculature in post-ischaemic disease, for their delivery to ischaemic tissues and for their assessment in pre-clinical and clinical studies:

- Although murine models have underpinned a wealth of basic biology studies, they also have certain limitations (reviewed extensively above). Standardization of animal models for cardiovascular research and inclusion of comorbidities are necessary to reach the standard for clinical translation. It is our view that large animal models including novel transgenic pig models, can be useful for long-term experimentation because their close similarity with human size, anatomy and metabolism enhances their relevance for clinical translation.
- Tissue specific delivery of pro-angiogenic therapies is advantageous, because it avoids the potential deleterious side effects associated with systemic delivery of growth factors such as the promotion of atherosclerosis. In the setting of PAD or coronary artery disease, local cell or gene therapy to promote post-ischaemic angiogenesis could be combined with systemic pharmacological therapy to reduce risk factors for atherosclerosis. A new generation of vectors should be developed to allow precise temporal control of inducible transgene expression, thus avoiding detrimental effects due to continuous overexpression.
- Endpoints of clinical trials of therapeutic vascularization have varied between studies. We propose that functional, metabolic, and imaging readouts should be further developed to capture therapeutic efficacy and biological activity of treatments, thereby support clinical hard endpoints.
- Patient selection is critical, given the influence that comorbidities, aging and medications may have on the results of the trials. Since safety of

gene and cell therapy has been very good in almost all reported trials, moving towards trials of less severe patients, such as Canadian Cardiovascular Society (CCS) Class 2–3 for refractory angina, in the future will be justified. Finally, further genetic characterization of non-responder patient groups in neovascularization clinical trials would help to identify factors affecting treatment responsiveness.

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References

- Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 1995;**11**:73–91.
- Coffin JD, Poole TJ. Embryonic vascular development: immunohistochemical identification of the origin and subsequent morphogenesis of the major vessel primordia in quail embryos. *Development* 1988;**102**:735–748.
- Poole TJ, Coffin JD. Vasculogenesis and angiogenesis: two distinct morphogenetic mechanisms establish embryonic vascular pattern. *J Exp Zool* 1989;**251**:224–231.
- Drake CJ, Fleming PA. Vasculogenesis in the day 6.5 to 9.5 mouse embryo. *Blood* 2000;**95**:1671–1679.
- Walls JR, Coultas L, Rossant J, Henkelman RM. Three-dimensional analysis of vascular development in the mouse embryo. *PLoS One* 2008;**3**:e2853.
- Brown LA, Rodaway AR, Schilling TF, Jowett T, Ingham PW, Patient RK, Sharrocks AD. Insights into early vasculogenesis revealed by expression of the ETS-domain transcription factor Fli-1 in wild-type and mutant zebrafish embryos. *Mech Dev* 2000;**90**:237–252.
- Jin SW, Beis D, Mitchell T, Chen JN, Stainier DY. Cellular and molecular analyses of vascular tube and lumen formation in zebrafish. *Development* 2005;**132**:5199–5209.
- Ellertsdottir E, Lenard A, Blum Y, Krudewig A, Hervig L, Affolter M, Belting HG. Vascular morphogenesis in the zebrafish embryo. *Dev Biol* 2010;**341**:56–65.
- Krebs LT, Shutter JR, Tanigaki K, Honjo T, Stark KL, Gridley T. Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev* 2004;**18**:2469–2473.
- Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, Weinstein BM. Notch signaling is required for arterial-venous differentiation during embryonic vascular development. *Development* 2001;**128**:3675–3683.
- Lawson ND, Vogel AM, Weinstein BM. Sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. *Dev Cell* 2002;**3**:127–136.
- Gritz E, Hirschi KK. Specification and function of hemogenic endothelium during embryogenesis. *Cell Mol Life Sci* 2016;**73**:1547–1567.
- Romagnani P, Annunziato F, Liotta F, Lazzeri E, Mazzinghi B, Frosali F, Cosmi L, Maggi L, Lasagni L, Scheffold A, Kruger M, Dimmeler S, Marra F, Gensini G, Maggi E, Romagnani S. CD14+CD34low cells with stem cell phenotypic and functional features are the major source of circulating endothelial progenitors. *Circ Res* 2005;**97**:314–322.
- Fernández-Avilés F, Sanz-Ruiz R, Climent AM, Badimon L, Bolli R, Charron D, Fuster V, Janssens S, Kastrup J, Kim HS, Lüscher TF, Martin JF, Menasché P, Simari RD, Stone GW, Terzic A, Willerson JT, Wu JC; TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) Writing Group; Authors/Task Force Members. Chairpersons; Basic Research Subcommittee; Translational Research Subcommittee; Challenges of Cardiovascular Regenerative Medicine Subcommittee; Tissue Engineering Subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee; Clinical Trials Subcommittee; Regulatory and funding strategies subcommittee; Delivery, Navigation, Tracking and Assessment Subcommittee. Global position paper on cardiovascular regenerative medicine. *Eur Heart J* 2017;**38**:2532–2546.
- Risau W. Mechanisms of angiogenesis. *Nature* 1997;**386**:671–674.
- Rissanen TT, Korpisalo P, Markkanen JE, Liimatainen T, Ordén M-R, Kholová I, de Goede A, Heikura T, Gröhn OH, Ylä-Herttuala S. Blood flow remodels growing vasculature during vascular endothelial growth factor gene therapy and determines between capillary arterIALIZATION and sprouting angiogenesis. *Circulation* 2005;**112**:3937–3946.
- Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 2003;**161**:1163–1177.
- Hasan SS, Tsaryk R, Lange M, Wisniewski L, Moore JC, Lawson ND, Wojciechowska K, Schnittler H, Siekmann AF. Endothelial Notch signalling limits angiogenesis via control of artery formation. *Nat Cell Biol* 2017;**19**:928–940.
- Pitulescu ME, Schmidt I, Gaiaro BD, Antoine T, Berkenfeld F, Ferrante F, Park H, Ehling M, Biljes D, Rocha SF, Langen UH, Stehling M, Nagasawa T, Ferrara N, Borggrete T, Adams RH. Dll4 and Notch signalling couples sprouting angiogenesis and artery formation. *Nat Cell Biol* 2017;**19**:915–927.
- Chouinard-Pelletier G, Jahnsen ED, Jones EA. Increased shear stress inhibits angiogenesis in veins and not arteries during vascular development. *Angiogenesis* 2013;**16**:71–83.
- Mahmoud MM, Kim HR, Xing R, Hsiao S, Mammoto A, Chen J, Serbanovic-Canic J, Feng S, Bowden NP, Maguire R, Arianas M, Francis SE, Weinberg PD, van der Heiden K, Jones EA, Chico TJ, Ridger V, Evans PC. TWIST1 integrates endothelial responses to flow in vascular dysfunction and atherosclerosis. *Circ Res* 2016;**119**:450–462.
- Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000;**6**:389–395.
- Silvestre JS, Smadja DM, Levy BI. Postischemic revascularization: from cellular and molecular mechanisms to clinical applications. *Physiol Rev* 2013;**93**:1743–1802.
- Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell* 2012;**148**:399–408.
- Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010;**140**:883–899.
- Costa C, Incio J, Soares R. Angiogenesis and chronic inflammation: cause or consequence? *Angiogenesis* 2007;**10**:149–166.
- Hoefler IE, den Adel B, Daemen MJ. Biomechanical factors as triggers of vascular growth. *Cardiovasc Res* 2013;**99**:276–283.
- Feng S, Bowden N, Fragiadakis M, Souilhol C, Hsiao S, Mahmoud M, Allen S, Pirri D, Ayllon BT, Akhtar S, Thompson AAR, Jo H, Weber C, Ridger V, Schober A, Evans PC. Mechanical activation of hypoxia-inducible factor 1α drives endothelial dysfunction at atheroprone sites. *Arterioscler Thromb Vasc Biol* 2017;**37**:2087–2101.
- Ghaffari S, Leask RL, Jones EA. Flow dynamics control the location of sprouting and direct elongation during developmental angiogenesis. *Development* 2015;**142**:4151–4157.
- Ghaffari S, Leask RL, Jones EAV. Blood flow can signal during angiogenesis not only through mechanotransduction, but also by affecting growth factor distribution. *Angiogenesis* 2017;**20**:373–384.
- Song JW, Munn LL. Fluid forces control endothelial sprouting. *Proc Natl Acad Sci USA* 2011;**108**:15342–15347.
- Dragneva G, Korpisalo P, Ylä-Herttuala S. Promoting blood vessel growth in ischemic diseases: challenges in translating preclinical potential into clinical success. *Dis Model Mech* 2013;**6**:312–322.
- Buerk DG, Goldstick TK. Arterial wall oxygen consumption rate varies spatially. *Am J Physiol* 1982;**243**:H948–H958.
- Gössl M, Versari D, Lerman LO, Chade AR, Beighley PE, Erbel R, Ritman EL. Low vasa vasorum densities correlate with inflammation and subintimal thickening: potential role in location-determination of atherogenesis. *Atherosclerosis* 2009;**206**:362–368.
- Sluimer JC, Gasc J-M, van Wanroij JL, Kisters N, Groeneweg M, Sollewijn Gelpke MD, Cleutjens JP, van den Akker LH, Corvol P, Wouters BG, Daemen MJ, Bijnens A-PJ. Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis. *J Am Coll Cardiol* 2008;**51**:1258–1265.
- Najafi AH, Agihli N, Tilan JU, Andrews JA, Peng X, Lassance-Soares RM, Sood S, Alderman LO, Abe K, Li L, Kolodgie FD, Virmani R, Zukowska Z, Epstein SE, Burnett MS. A new murine model of stress-induced complex atherosclerotic lesions. *Dis Model Mech* 2013;**6**:323–331.
- Roth L, Rombouts M, Schrijvers DM, Lemmens K, De Keulenaer GW, Martinet W, De Meyer GRY. Chronic intermittent mental stress promotes atherosclerotic plaque vulnerability, myocardial infarction and sudden death in mice. *Atherosclerosis* 2015;**242**:288–294.
- Haghighat A, Weiss D, Whalin MK, Cowan DP, Taylor WR. Granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor exacerbate atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2007;**115**:2049–2054.

39. Celletti FL, Waugh JM, Amabile PG, Brendolan A, Hilfiker PR, Dake MD. Vascular endothelial growth factor enhances atherosclerotic plaque progression. *Nat Med* 2001;**7**:425–429.
40. Lucerna M, Zerneck A, de Nooijer R, de Jager SC, Bot I, van der Lans C, Kholova I, Liehn EA, van Berkel TJC, Yla-Herttuala S, Weber C, Biessen EAL. Vascular endothelial growth factor-A induces plaque expansion in ApoE knock-out mice by promoting de novo leukocyte recruitment. *Blood* 2007;**109**:122–129.
41. Bhardwaj S, Roy H, Heikura T, Ylä-Herttuala S. VEGF-A, VEGF-D and VEGF-D(DeltaNDeltaC) induced intimal hyperplasia in carotid arteries. *Eur J Clin Invest* 2005;**35**:669–676.
42. Khurana R, Zhuang Z, Bhardwaj S, Murakami M, De Muinck E, Yla-Herttuala S, Ferrara N, Martin JF, Zachary I, Simons M. Angiogenesis-dependent and independent phases of intimal hyperplasia. *Circulation* 2004;**110**:2436–2443.
43. Giannarelli C, Alique M, Rodriguez DT, Yang DK, Jeong D, Calcagno C, Hutter R, Millon A, Kovacic JC, Weber T, Faries PL, Soff GA, Fayad ZA, Hajjar RJ, Fuster V, Badimon JJ. Alternatively spliced tissue factor promotes plaque angiogenesis through the activation of hypoxia-inducible factor-1 α and vascular endothelial growth factor signaling. *Circulation* 2014;**130**:1274–1286.
44. Leppänen P, Koota S, Kholová I, Koponen J, Fieber C, Eriksson U, Alitalo K, Ylä-Herttuala S. Gene transfers of vascular endothelial growth factor-A, vascular endothelial growth factor-B, vascular endothelial growth factor-C, and vascular endothelial growth factor-D have no effects on atherosclerosis in hypercholesterolemic low-density lipoprotein. *Circulation* 2005;**112**:1347–1352.
45. Sluimer JC, Kolodgie FD, Bijnens AP, Maxfield K, Pacheco E, Kutys B, Duimel H, Frederik PM, van Hinsbergh VW, Virmani R, Daemen MJ. Thin-walled microvessels in human coronary atherosclerotic plaques show incomplete endothelial junctions: relevance of compromised structural integrity for intraplaque microvascular leakage. *J Am Coll Cardiol* 2009;**53**:1517–1527.
46. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003;**9**:653–660.
47. Virmani R, Kolodgie FD, Burke AP, Finn AV, Gold HK, Tulenko TN, Wrenn SP, Narula J. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol* 2005;**25**:2054–2061.
48. Eriksson EE. Intravital microscopy on atherosclerosis in apolipoprotein e-deficient mice establishes microvessels as major entry pathways for leukocytes to advanced lesions. *Circulation* 2011;**124**:2129–2138.
49. Rademakers T, Douma K, Hackeng TM, Post MJ, Sluimer JC, Daemen MJAP, Biessen EAL, Heeneman S, van Zandvoort MAMJ. Plaque-associated vasa vasorum in aged apolipoprotein E-deficient mice exhibit proatherogenic functional features in vivo. *Arterioscler Thromb Vasc Biol* 2013;**33**:249–256.
50. Michel J-B, Martin-Ventura JL, Nicoletti A, Ho-Tin-Noé B. Pathology of human plaque vulnerability: mechanisms and consequences of intraplaque haemorrhages. *Atherosclerosis* 2014;**234**:311–319.
51. Choi JS, Kim KB, Han W, Kim DS, Park JS, Lee JJ, Lee DS. Efficacy of therapeutic angiogenesis by intramyocardial injection of pCK-VEGF165 in pigs. *Ann Thorac Surg* 2006;**82**:679–686.
52. Horvath KA, Doukas J, Lu CY, Belkind N, Greene R, Pierce GF, Fullerton DA. Myocardial functional recovery after fibroblast growth factor 2 gene therapy as assessed by echocardiography and magnetic resonance imaging. *Ann Thorac Surg* 2002;**74**:481–486; discussion 487.
53. Taniyama Y, Morishita R, Aoki M, Nakagami H, Yamamoto K, Yamazaki K, Matsumoto K, Nakamura T, Kaneda Y, Ogihara T. Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat and rabbit hindlimb ischemia models: preclinical study for treatment of peripheral arterial disease. *Gene Ther* 2001;**8**:181–189.
54. Takahashi K, Ito Y, Morikawa M, Kobune M, Huang J, Tsukamoto M, Sasaki K, Nakamura K, Dehari H, Ikeda K, Uchida H, Hirai S, Abe T, Hamada H. Adenoviral-delivered angiopoietin-1 reduces the infarction and attenuates the progression of cardiac dysfunction in the rat model of acute myocardial infarction. *Mol Ther* 2003;**8**:584–592.
55. Dobrucki LW, Tsutsumi Y, Kalinowski L, Dean J, Gavin M, Sen S, Mendizabal M, Sinusas AJ, Aikawa R. Analysis of angiogenesis induced by local IGF-1 expression after myocardial infarction using microSPECT-CT imaging. *J Mol Cell Cardiol* 2010;**48**:1071–1079.
56. Yla-Herttuala S, Baker AH. Cardiovascular gene therapy: past, present, and future. *Mol Ther* 2017;**25**:1095–1106.
57. Yla-Herttuala S, Rissanen TT, Vajanto I, Hartikainen J. Vascular endothelial growth factors: biology and current status of clinical applications in cardiovascular medicine. *J Am Coll Cardiol* 2007;**49**:1015–1026.
58. Devesa L, Choi J, Yang F. Therapeutic angiogenesis for treating cardiovascular diseases. *Theranostics* 2012;**2**:801–814.
59. Yu P, Wilhelm K, Dubrac A, Tung JK, Alves TC, Fang JS, Xie Y, Zhu J, Chen Z, De Smet F, Zhang J, Jin SW, Sun L, Sun H, Kibbey RG, Hirschi KK, Hay N, Carmeliet P, Chittenden TV, Eichmann A, Potente M, Simons M. FGF-dependent metabolic control of vascular development. *Nature* 2017;**545**:224–228.
60. Losordo DW, Dimmeler S. Therapeutic angiogenesis and vasculogenesis for ischemic disease. Part I: angiogenic cytokines. *Circulation* 2004;**109**:2487–2491.
61. Ho TK, Shiwen X, Abraham D, Tsui J, Baker D. Stromal-cell-derived factor-1 (SDF-1)/CXCL12 as potential target of therapeutic angiogenesis in critical leg ischaemia. *Cardiol Res Pract* 2012;**2012**:143209.
62. Zhang S, Ge J, Zhao L, Qian J, Huang Z, Shen L, Sun A, Wang K, Zou Y. Host vascular niche contributes to myocardial repair induced by intracoronary transplantation of bone marrow CD34+ progenitor cells in infarcted swine heart. *Stem Cells* 2007;**25**:1195–1203.
63. Williams AR, Suncion VY, McCall F, Guerra D, Mather J, Zambrano JP, Heldman AW, Hare JM. Durable scar size reduction due to allogeneic mesenchymal stem cell therapy regulates whole-chamber remodeling. *J Am Heart Assoc* 2013;**2**:e000140.
64. Kulandavelu S, Karantalis V, Fritsch J, Hatzistergos KE, Loescher VY, McCall F, Wang B, Bagnoli L, Golpanian S, Wolf A, Grenet J, Williams A, Kupin A, Rosenfeld A, Mohsin S, Sussman MA, Morales A, Balkan VW, Hare JM. Pim1 kinase overexpression enhances ckit(+) cardiac stem cell cardiac repair following myocardial infarction in swine. *J Am Coll Cardiol* 2016;**68**:2454–2464.
65. Hou L, Kim JJ, Woo YJ, Huang NF. Stem cell-based therapies to promote angiogenesis in ischemic cardiovascular disease. *Am J Physiol Heart Circ Physiol* 2016;**310**:H455–H465.
66. Raval Z, Losordo DW. Cell therapy of peripheral arterial disease: from experimental findings to clinical trials. *Circ Res* 2013;**112**:1288–1302.
67. Samura M, Hosoyama T, Takeuchi Y, Ueno K, Morikage N, Hamano K. Therapeutic strategies for cell-based neovascularization in critical limb ischemia. *J Transl Med* 2017;**15**:49.
68. Poller W, Dimmeler S, Heymans S, Zeller T, Haas J, Karakas M, Leistner DM, Jakob P, Nakagawa S, Blankenberg S, Engelhardt S, Thum T, Weber C, Meder B, Hajjar R, Landmesser U. Non-coding RNAs in cardiovascular diseases: diagnostic and therapeutic perspectives. *Eur Heart J* 2017;doi:10.1093/eurheartj/ehx165.
69. Schober A, Nazari-Jahanigh M, Wei Y, Bidzhikov K, Gremse J, Grommes J, Megens RT, Heyll K, Noels H, Hristov M, Wang S, Kiessling F, Olson EN, Weber C. MicroRNA-126-5p promotes endothelial proliferation and limits atherosclerosis by suppressing Dlk1. *Nat Med* 2014;**20**:368–376.
70. Zerneck A, Bidzhikov K, Noels H, Shagdarsuren E, Gan L, Denecke B, Hristov M, Koppel T, Jahanigh MN, Lutgens E, Wang S, Olson EN, Schober A, Weber C. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. *Sci Signal* 2009;**2**:ra81.
71. Landskroner-Eiger S, Qiu C, Perrotta P, Siragusa M, Lee MY, Ulrich V, Luciano AK, Zhuang ZW, Corti F, Simons M, Montgomery RL, Wu D, Yu J, Sessa WC. Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling. *Proc Natl Acad Sci USA* 2015;**112**:12812–12817.
72. Caporali A, Meloni M, Vollenkle C, Bonci D, Sala-Newby GB, Addis R, Spinetti G, Losa S, Masson R, Baker AH, Agami R, Le Sage C, Condorelli G, Madeddu P, Martelli F, Emanueli C. Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. *Circulation* 2011;**123**:282–291.
73. Mellis D, Caporali A. MicroRNA-based therapeutics in cardiovascular disease: screening and delivery to the target. *Biochem Soc Trans* 2017;**46**:11–21.
74. Yla-Herttuala S, Bridges C, Katz MG, Korpisalo P. Angiogenic gene therapy in cardiovascular diseases: dream or vision? *Eur Heart J* 2017;**38**:1365–1371.
75. Pajusola K, Kunnappu J, Vuorikoski S, Soronen J, Andre H, Pereira T, Korpisalo P, Yla-Herttuala S, Poellinger L, Alitalo K. Stabilized HIF-1 α is superior to VEGF for angiogenesis in skeletal muscle via adeno-associated virus gene transfer. *FASEB J* 2005;**19**:1365–1367.
76. Fisher SA, Doree C, Taggart DP, Mathur A, Martin-Rendon E. Cell therapy for heart disease: trial sequential analyses of two Cochrane reviews. *Clin Pharmacol Ther* 2016;**100**:88–101.
77. Banovic M, Loncar Z, Behfar A, Vanderheyden M, Beleslin B, Zeiher A, Metra M, Terzic A, Bartunek J. Endpoints in stem cell trials in ischemic heart failure. *Stem Cell Res Ther* 2015;**6**:159.
78. Hassinen I, Kivela A, Hedman A, Saraste A, Knuuti J, Hartikainen JE, Yla-Herttuala S. Intramyocardial gene therapy directed to hibernating heart muscle using a combination of electromechanical mapping and positron emission tomography. *Hum Gene Ther* 2016;**27**:830–834.
79. Merentie M, Lottonen-Raikaslehto L, Parviainen V, Huusko J, Pikkariainen S, Mendel M, Laham-Karam N, Kärjä V, Rissanen R, Hedman M, Yla-Herttuala S. Efficacy and safety of myocardial gene transfer of adenovirus, adeno-associated virus and lentivirus vectors in the mouse heart. *Gene Ther* 2016;**23**:296–305.
80. Hulot JS, Ishikawa K, Hajjar RJ. Gene therapy for the treatment of heart failure: promise postponed. *Eur Heart J* 2016;**37**:1651–1658.
81. Park HJ, Yang F, Cho SW. Nonviral delivery of genetic medicine for therapeutic angiogenesis. *Adv Drug Deliv Rev* 2012;**64**:40–52.
82. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. *Nat Rev Drug Discov* 2005;**4**:581–593.
83. Jeon O, Yang HS, Lee TJ, Kim BS. Heparin-conjugated polyethylenimine for gene delivery. *J Control Release* 2008;**132**:236–242.
84. Kang SW, Lim HW, Seo SW, Jeon O, Lee M, Kim BS. Nanosphere-mediated delivery of vascular endothelial growth factor gene for therapeutic angiogenesis in mouse ischemic limbs. *Biomaterials* 2008;**29**:1109–1117.

85. Kumagai M, Marui A, Tabata Y, Takeda T, Yamamoto M, Yonezawa A, Tanaka S, Yanagi S, Ito-Ihara T, Ikeda T, Murayama T, Teramukai S, Katsura T, Matsubara K, Kawakami K, Yokode M, Shimizu A, Sakata R. Safety and efficacy of sustained release of basic fibroblast growth factor using gelatin hydrogel in patients with critical limb ischemia. *Heart Vessels* 2016;**31**:713–721.
86. Morales C, Gonzalez GE, Rodriguez M, Bertolasi CA, Gelpi RJ. Histopathologic time course of myocardial infarct in rabbit hearts. *Cardiovasc Pathol* 2002;**11**:339–345.
87. Rissanen TT, Markkanen JE, Arve K, Rutanen J, Kettunen MI, Vajanto I, Jauhainen S, Cashion L, Gruchala M, Narvanen O, Taipale P, Kauppinen RA, Rubanyi GM, Yla HS. Fibroblast growth factor 4 induces vascular permeability, angiogenesis and arteriogenesis in a rabbit hindlimb ischemia model. *FASEB J* 2003;**17**:100–102.
88. Hinkel R, Lebherz C, Fydanaki M, Wucher A, El-Aouni C, Thormann M, Thein E, Kupatt C, Boekstegers P. Angiogenic potential of Ad2/Hif-1alpha/VP16 after regional application in a preclinical pig model of chronic ischemia. *Curr Vasc Pharmacol* 2013;**11**:29–37.
89. Rissanen TT, Nurro J, Halonen PJ, Tarkia M, Saraste A, Rannankari M, Honkonen K, Pietila M, Leppanen O, Kuivaniemi A, Knuuti J, Yla-Herttuala S. The bottleneck stent model for chronic myocardial ischemia and heart failure in pigs. *Am J Physiol Heart Circ Physiol* 2013;**305**:H1297–H1308.
90. Iwanaga K, Takano H, Ohtsuka M, Hasegawa H, Zou Y, Qin Y, Odaka K, Hiroshima K, Tadokoro H, Komuro I. Effects of G-CSF on cardiac remodeling after acute myocardial infarction in swine. *Biochem Biophys Res Commun* 2004;**325**:1353–1359.
91. Buschmann IR, Voskuil M, van Royen N, Hoefer IE, Scheffler K, Grundmann S, Hennig J, Schaper W, Bode C, Piek JJ. Invasive and non-invasive evaluation of spontaneous arteriogenesis in a novel porcine model for peripheral arterial obstructive disease. *Atherosclerosis* 2003;**167**:33–43.
92. Unger EF, Banai S, Shou M, Jaklitsch M, Hodge E, Correa R, Jaye M, Epstein SE. A model to assess interventions to improve collateral blood flow: continuous administration of agents into the left coronary artery in dogs. *Cardiovasc Res* 1993;**27**:785–791.
93. Unger EF, Banai S, Shou M, Lazarous DF, Jaklitsch MT, Scheinowitz M, Correa R, Klingbeil C, Epstein SE. Basic fibroblast growth factor enhances myocardial collateral flow in a canine model. *Am J Physiol* 1994;**266**:H1588–H1595.
94. Marques SM, Campos PP, Castro PR, Cardoso CC, Ferreira MA, Andrade SP. Genetic background determines mouse strain differences in inflammatory angiogenesis. *Microvasc Res* 2011;**82**:246–252.
95. Niiyama H, Huang NF, Rollins MD, Cooke JP. Murine model of hindlimb ischemia. *J Vis Exp* 2009;1035.
96. Vinegoni C, Aguirre AD, Lee S, Weissleder R. Imaging the beating heart in the mouse using intravital microscopy techniques. *Nat Protoc* 2015;**10**:1802–1819.
97. Cambria E, Pasqualini FS, Wolint P, Gunter J, Steiger J, Bopp A, Hoerstrup SP, Emmert MY. Translational cardiac stem cell therapy: advancing from first-generation to next-generation cell types. *NPJ Regen Med* 2017;**2**:17.
98. O'Connor JP, Aboagye EO, Adams JE, Aerts HJ, Barrington SF, Beer AJ, Boellaard R, Bohndiek SE, Brady M, Brown G, Buckley DL, Chenevert TL, Clarke LP, Collette S, Cook GJ, deSouza NM, Dickson JC, Dive C, Evelhoch JL, Faivre-Finn C, Gallagher FA, Gilbert FJ, Gillies RJ, Goh V, Griffiths JR, Groves AM, Halligan S, Harris AL, Hawkes DJ, Hoekstra OS, Huang EP, Hutton BF, Jackson EF, Jayson GC, Jones A, Koh DM, Lacombe D, Lambin P, Lassar M, Leach MO, Lee TY, Leen EL, Lewis JS, Liu Y, Lythgoe MF, Manoharan P, Maxwell RJ, Miles KA, Morgan B, Morris S, Ng T, Padhani AR, Parker GJ, Partridge M, Pathak AP, Peet AC, Punwani S, Reynolds AR, Robinson SP, Shankar LK, Sharma RA, Soloviev D, Stroobants S, Sullivan DC, Taylor SA, Tofts PS, Tozer GM, van Herk M, Walker-Samuel S, Wason J, Williams KJ, Workman P, Yankeelov TE, Brindle KM, McShane LM, Jackson A, Waterton JC. Imaging biomarker roadmap for cancer studies. *Nat Rev Clin Oncol* 2017;**14**:169–186.
99. Jøkerst JV, Cauwenberghs N, Kuznetsova T, Haddad F, Sweeney T, Hou J, Rosenberg-Hasson Y, Zhao E, Schutt R, Bolli R, Traverse JH, Pepine CJ, Henry TD, Schulman IH, Moye L, Taylor DA, Yang PC. Circulating biomarkers to identify responders in cardiac cell therapy. *Sci Rep* 2017;**7**:4419.
100. Yla-Herttuala S, Bridges C, Katz MG, Korpisalo P. Angiogenic gene therapy in cardiovascular diseases: dream or vision? *Eur Heart J* 2017;**38**:1365–1371.
101. Rigato M, Monami M, Fadini GP. Autologous cell therapy for peripheral arterial disease: systematic review and meta-analysis of randomized, nonrandomized, and non-controlled studies. *Circ Res* 2017;**120**:1326–1340.
102. Hartikainen J, Hassinen I, Hedman A, Kivela A, Saraste A, Knuuti J, Husso M, Mussalo H, Hedman M, Rissanen TT, Toivanen P, Heikura T, Witztum JL, Tsimikas S, Yla-Herttuala S. Adenoviral intramyocardial VEGF-DΔNΔC gene transfer increases myocardial perfusion reserve in refractory angina patients: a phase I/IIa study with 1-year follow-up. *Eur Heart J* 2017;**38**:2547–2555.
103. Sedding DG, Boyle EC, Demandt JAF, Sluimer JC, Dutzmann J, Haverich A, Bauersachs J. Vasa vasorum angiogenesis: key player in the initiation and progression of atherosclerosis and potential target for the treatment of cardiovascular disease. *Front Immunol* 2018;**9**:706.
104. Kampschulte M, Gunkel I, Stieger P, Sedding DG, Brinkmann A, Ritman EL, Krombach GA, Langheinrich AC. Thalidomide influences atherogenesis in aortas of ApoE(-/-)/LDLR(-/-) double knockout mice: a nano-CT study. *Int J Cardiovasc Imaging* 2014;**30**:795–802.
105. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski WW, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. *Circulation* 1999;**99**:1726–1732.
106. Moulton KS, Olsen BR, Sonn S, Fukai N, Zurakowski D, Zeng X. Loss of collagen XVIII enhances neovascularization and vascular permeability in atherosclerosis. *Circulation* 2004;**110**:1330–1336.
107. Stefanadis C, Toutouzas K, Stefanadi E, Lazaris A, Patsouris E, Kipshidze N. Inhibition of plaque neovascularization and intimal hyperplasia by specific targeting vascular endothelial growth factor with bevacizumab-eluting stent: an experimental study. *Atherosclerosis* 2007;**195**:269–276.
108. Hauer AD, Habets KLL, van Wanrooij EJA, de Vos P, Krueger J, Reisfeld RA, van Berkel TJC, Kuiper J. Vaccination against TIE2 reduces atherosclerosis. *Atherosclerosis* 2009;**204**:365–371.
109. Hauer AD, van Puijvelde GHM, Peterse N, de Vos P, van Weel V, van Wanrooij EJA, Biessen EAL, Quax PHA, Niethammer AG, Reisfeld RA, van Berkel TJC, Kuiper J. Vaccination against VEGFR2 attenuates initiation and progression of atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007;**27**:2050–2057.
110. Xu X, Mao W, Chai Y, Dai J, Chen Q, Wang L, Zhuang Q, Pan Y, Chen M, Ni G, Huang Z. Angiogenesis inhibitor, endostatin, prevents vasa vasorum neovascularization in a swine atherosclerosis model. *J Atheroscler Thromb* 2015;**22**:1100–1112.
111. Girardi F, Franceschi E, Brandes AA. Cardiovascular safety of VEGF-targeting therapies: current evidence and handling strategies. *Oncologist* 2010;**15**:683–694.
112. Van der Donck C, Van Herck JL, Schrijvers DM, Vanhoutte G, Verhoye M, Blockx I, Van Der Linden A, Bauders D, Lijnen HR, Sluimer JC, Roth L, Van Hove CE, Franssen P, Knaapen MW, Hervent A-S, De Keulenaer GW, Bult H, Martinet W, Herman AG, De Meyer GRY. Elastin fragmentation in atherosclerotic mice leads to intraplaque neovascularization, plaque rupture, myocardial infarction, stroke, and sudden death. *Eur Heart J* 2015;**36**:1049–1058.
113. Tanaka K, Nagata D, Hirata Y, Tabata Y, Nagai R, Sata M. Augmented angiogenesis in adventitia promotes growth of atherosclerotic plaque in apolipoprotein E-deficient mice. *Atherosclerosis* 2011;**215**:366–373.
114. Chen Y, Song YX, Wang ZN. The microRNA-148/152 family: multi-faceted players. *Mol Cancer* 2013;**12**:43.
115. Camare C, Pucelle M, Negre-Salvayre A, Salvayre R. Angiogenesis in the atherosclerotic plaque. *Redox Biol* 2017;**12**:18–34.
116. Riou LM, Boisat A, Ghezzi C, Finet G, Rioufol G, Gharib AM, Pettigrew RI, Ohayon J. Effects of mechanical properties and atherosclerotic artery size on biomechanical plaque disruption—mouse vs. human. *J Biomech* 2014;**47**:765–772.
117. Van der Heiden K, Hoogendoorn A, Daemen MJ, Gijzen FJH. Animal models for plaque rupture: a biomechanical assessment. *Thromb Haemostasis* 2016;**115**:501.
118. Rivron NC, Vrij EJ, Rouwkema J, Le Gac S, van den Berg A, Truckenmuller RK, van Blitterswijk CA. Tissue deformation spatially modulates VEGF signaling and angiogenesis. *Proc Natl Acad Sci USA* 2012;**109**:6886–6891.
119. Pelisek J, Well G, Reeps C, Rudelius M, Kuehn L, Culmes M, Poppert H, Zimmermann A, Berger H, Eckstein H-H. Neovascularization and angiogenic factors in advanced human carotid artery stenosis. *Circ J* 2012;**76**:1274–1282.
120. Kumamoto M, Nakashima Y, Sueshiki K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. *Hum Pathol* 1995;**26**:450–456.
121. Stenmark KR, Yeager ME, El Kasm K, Nozik-Grayck E, Gerasimovskaya EV, Li M, Riddle SR, Frid MG. The adventitia: essential regulator of vascular wall structure and function. *Annu Rev Physiol* 2013;**75**:23–47.
122. Petrovan RJ, Kaplan CD, Reisfeld RA, Curtiss LK. DNA vaccination against VEGF receptor 2 reduces atherosclerosis in LDL receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2007;**27**:1095–1100.
123. Bhardwaj S, Roy H, Babu M, Shibuya M, Yla-Herttuala S. Adventitial gene transfer of VEGFR-2 specific VEGF-E chimera induces MCP-1 expression in vascular smooth muscle cells and enhances neointimal formation. *Atherosclerosis* 2011;**219**:84–91.
124. Roy H, Bhardwaj S, Babu M, Kokina I, Uotila S, Ahtialansaari T, Laitinen T, Hakumaki J, Laakso M, Herzig KH, Yla-Herttuala S. VEGF-A, VEGF-D, VEGF receptor-1, VEGF receptor-2, NF-kappaB, and RAGE in atherosclerotic lesions of diabetic Watanabe heritable hyperlipidemic rabbits. *FASEB J* 2006;**20**:2159–2161.
125. Al-Mashhadi RH, Sorensen CB, Kragh PM, Christoffersen C, Mortensen MB, Tolbod LP, Thim T, Du Y, Li J, Liu Y, Moldt B, Schmidt M, Vajta G, Larsen T, Purup S, Bolund L, Nielsen LB, Callesen H, Falk E, Mikkelsen JG, Bentzon JF. Familial hypercholesterolemia and atherosclerosis in cloned minipigs created by DNA transposition of a human PCSK9 gain-of-function mutant. *Sci Transl Med* 2013;**5**:166ra1.
126. Bhardwaj S, Roy H, Kärpänä T, He Y, Hi Y, Jauhainen S, Hedman M, Alitalo K, Yla-Herttuala S. Periadventitial angiopoietin-1 gene transfer induces angiogenesis in rabbit carotid arteries. *Gene Ther* 2005;**12**:388–394.